Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding

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Abstract: The effects of controlled feeding versus starvation during quarantine on mantle tissue glycogen concentration (represented as milligrams glycogen per gram of mantle tissue ± SD) of two freshwater mussel species were compared. Starved individuals were not provided with supplemental food during quarantine, while fed specimens were provided with 10^5 algal cells/ml, twice per day. Initial mean glycogen levels for *Amblema plicata* (Say, 1817) (9.4 ± 2.4 mg/g) and *Quadrula pustulosa* (I. Lea, 1831) (7.9 ± 1.8 mg/g) collected from Ohio River Mile (ORM) 175.5 in July 1997 were not significantly different (p > 0.3) from mean glycogen levels of *A. plicata* (8.1 ± 4.2 mg/g) and *Q. pustulosa* (6.2 ± 2.9 mg/g) collected from the same site in July 1996. Initial glycogen concentrations of quarantined mussels, therefore, were similar in both the starved and fed groups. After seven days of feeding in quarantine, mean glycogen levels of *A. plicata* (12.3 ± 2.3 mg/g) and *Q. pustulosa* (7.1 ± 3.7 mg/g) did not change significantly (p > 0.1) relative to wild-caught specimens, and were significantly larger (p < 0.05) than mean glycogen levels of starved individuals (3.6 ± 1.8 mg/g and 3.5 ± 2.3 mg/g, respectively). Similarly, mean glycogen levels of *A. plicata* and *Q. pustulosa* after 14 days (8.1 ± 3.3 mg/g and 7.7 ± 3.3 mg/g, respectively) and 30 days (9.9 ± 4.8 mg/g and 8.4 ± 2.7 mg/g, respectively) of feeding were significantly larger (p < 0.01) than mean glycogen levels of starved specimens after 14 days (3.27 ± 1.74 mg/g and 5.37 ± 3.06 mg/g, respectively) and 30 days (1.2 ± 0.5 mg/g and 1.9 ± 1.4 mg/g, respectively). Adequate feeding of unionids in quarantine is essential to maintain animals in a condition that will increase the likelihood of survival following relocation.

Key Words: glycogen, zebra mussels, Ohio River, quarantine, Dreissenidae

Relocation has been used widely as a management tool to re-establish or assist in the recovery of declining populations of terrestrial and aquatic organisms (Griffith *et al.*, 1989; Cope and Waller, 1995). In response to recent declines in the unionid fauna of North America (Williams *et al.,* 1993), relocation is being tested as a management technique to (1) re-introduce extirpated populations, (2) remove unionids from project construction zones, (3) augment populations to increase genetic diversity, and (4) salvage unionids from zebra mussel-infested areas (Cope and Waller, 1995). The ultimate goal of any relocation project is to establish a self-sustaining population of the target organism, and several authors have developed a list of factors that should extend population persistence after relocation, including (1) the presence of suitable habitat and refugia, (2) the release of large founder groups with high genetic diversity, (3) low levels of competition, and (4) high rates of population growth (Griffith *et al.*, 1989; Cope and Waller, 1995). Little attention, however, has been given to the biochemical and physiological condition of an organism prior to relocation and its influence on survival and the ability to develop reproductively viable populations after relocation.

Condition, as defined by Mann (1978: 490), is the "ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological." Glycogen is the primary energy store in bivalves (Bayne, 1976; Gabbott, 1983), and the relative amount of glycogen stored in bivalve tissues is considered a good indicator of body condition (Galtsoff, 1964; Walne, 1970). Significant reductions in unionid glycogen stores prior to and during relocation, therefore, could greatly reduce their ability to cope with natural stressors present in the new environment.

Collection, handling, aerial exposure, abrupt temperature changes, holding, and transport of unionids during relocation can result in varying degrees of stress, depending on the length of exposure, that can in turn adversely affect body condition (Cope and Waller, 1995). Relocation of unionids to avoid the adverse effects of bridge construction, for example, could have little or no effect on condition if individual specimens are removed from their natural habitat for short time periods (several hours or days). The presence of zebra mussels, however, has been shown to cause decreased glycogen reserves in unionids (Haag *et al.*,

1993). In addition, zebra mussel-infested unionids presently require a 30-day quarantine period to ensure that zebra mussel adults and juveniles are removed prior to relocation (J. Clayton, pers. comm.). In addition to the stress of relocation, unionids can experience nutritive stress in quarantine because little or no information exists on their nutritional requirements (Gatenby et al., 1997). Under laboratory or hatchery conditions, bivalve energy stores have been shown to decline without proper feeding (Calvin, 1931; Pora et al., 1969; Bayne and Thompson, 1970; Gabbott and Walker, 1971), and recent studies reveal that unionid glycogen stores can decline as much as 80% after only 30 days of starvation in quarantine (Patterson et al., 1997). Consequently, the development of a feeding regime for mussels held in quarantine could be a critical link in the success of future relocation projects. The objectives of this experiment were to (1) monitor the glycogen levels of unionids during controlled feeding in quarantine, and (2) compare these results to reported changes in unionid glycogen levels during starvation in quarantine, as reported by Patterson et al. (1997).

METHODS

On 20 July 1997, ten specimens each of *Amblema plicata* (Say, 1817) and *Quadrula pustulosa* (I. Lea, 1831) were collected from Ohio River Mile (ORM) 175.5 near Parkersburg, West Virginia. Mussels were removed from the shell and placed in 95% ethanol for the determination of initial glycogen levels. Additional specimens of *A. plicata* and *Q. pustulosa* (N = 200 and 80, respectively) were collected from ORM 175.5 for placement in individual quarantine tanks. Unionids salvaged from zebra mussel-infested waters were thoroughly scrubbed to remove zebra mussels. Cleaned unionids were then hand-inspected before being placed in aerated quarantine tanks without substratum for a minimum of 30 days. During this 30-day period, water temperatures were maintained around 20°C to allow juvenile zebra mussels missed during the scrubbing procedure to become visible. At the end of 30 days, individual unionids were inspected under 10X magnification to ensure the absence of zebra mussels prior to relocation. Ten specimens of *A. plicata* and *Q. pustulosa* were sacrificed after seven, 14, and 30 days of quarantine, and preserved in 95% ethanol for subsequent glycogen analysis. After the experiment, all remaining specimens were returned to the Ohio River.

In 1997, unionids were fed cultures of the chlorophyte, *Neochloris oleoabundans* Chantanachat and Bold, 1962. This species was chosen because previous experiments indicated that juvenile unionids readily ingest and assimilate this alga (Gatenby et al., 1997). Initial stock cultures of *N. oleoabundans* were grown in Bold's Basal Medium (Nichols, 1973) under continuous cool white fluorescent light (photon flux: 60-100 $\mu E/m^2/s$) at 20°C. Stock cultures were then transferred to the quarantine facility and used to inoculate a 20 l carboy containing Fritz F2 algal medium (Fritz Aquaculture, Mesquite, Texas). When cell densities in the carboy reached 10^6 cells/ml, 7 l aliquots were used to inoculate three 250 l algal culture tanks (Aquatic Ecosystems, Inc., Apopka, Florida), that also were fertilized with Fritz F2 algal medium, aerated, and placed outside the quarantine facility in direct sunlight. Culture tanks were placed outside because algal cultures continually failed inside the quarantine facility, possibly due to insufficient light or high water temperatures. Algal cell densities in the 250 l culture tanks reached 10^6 cells/ml in *ca.* 4 days. Water samples from the culture tanks were collected daily and fixed with acid Lugol's solution (Saraceni and Ruggiu, 1969) for enumeration and identification of the algae. Water samples from the Ohio River in 1996 and 1997 showed that algal cell densities ranged from 10^4 - 10^5/cells/ml during the summer (Parker et al., 1998), thus unionids in quarantine were fed ca. 10^5 cells/ml twice per day, at 8:00 AM and 5:00 PM. Each day, 75% of the water in the quarantine tanks was drained; fresh water and food were added, and vigorous aeration was applied to maintain algal cells in suspension.

The glycogen content of all preserved specimens was determined from mantle tissue using the technique of Keppler and Decker (1974) as described in Patterson et al. (1997). Mean glycogen levels were expressed in milligrams of glycogen per gram of preserved mantle tissue ± standard deviation (SD). It should be noted that preserved tissue weights overestimate dry weights and underestimate wet tissue weights because 95% ethanol dehydrates tissue. Dehydration by 95% ethanol also reduces error that can result from changes in tissue water levels during stress. Mean glycogen levels were compared using ANOVA and, if significant differences were detected, the Scheffe F-test was used to determine the statistical significance of individual treatments.

RESULTS AND DISCUSSION

During the first 14 d of quarantine, *Neochloris oleoabundans* comprised > 95% of the algae in the 250-l culture containers. Because culture tanks were maintained outside the quarantine facility, cultures were contaminated with low densities of two green algae, *Scenedesmus* sp. and *Ankistrodesmus* sp. Once cultures were contaminated, densities of these contaminants continued to increase. After 30 d, the algal community in the culture containers had changed significantly, with *Scenedesmus* and

Ankistrodesmus comprising *ca.* 40% of the available algae, and *N. oleoabundans* comprising the remaining 60%. Despite changes in the algal community, cell densities remained at 10^6 cells/ml throughout the experiment.

Both the fed and starved treatments had some intial mortality likely as a result of collection and handling, however, no mortality occurred during the remainder of the quarantine period. Mean glycogen levels of *Amblema plicata* and *Quadrula pustulosa* during controlled feeding in quarantine remained the same, whereas mean glycogen levels declined in a previous starvation experiment by Patterson *et al.* (1997) (Fig. 1). Initial mean glycogen levels for *A. plicata* (9.4 ± 2.4 mg/g) and *Q. pustulosa* (7.9 ± 1.8 mg/g) collected from ORM 175.5 in July 1997 were not significantly different (p > 0.3) from the mean glycogen levels of *A. plicata* (8.1 ± 4.2 mg/g) and *Q. pustulosa* (6.2 ± 2.9 mg/g) collected from the same site in July 1996 (Patterson *et al.*, 1997). Glycogen stores of unionids entering quarantine, therefore, were similar in both the starvation and controlled feeding experiments. After seven days of feeding in quarantine, mean glycogen levels of *A. plicata* (12.3 ± 2.3 mg/g) and *Q. pustulosa* (7.1 ± 3.7 mg/g) did not change significantly (p > 0.1) relative to wild-caught specimens, and were significantly larger (p < 0.05) than mean glycogen levels of starved individuals (3.6 ± 1.8 mg/g and 3.5 ± 2.3 mg/g, respectively). Similarly, mean glycogen levels of *A. plicata* and *Q. pustulosa* after 14 days (8.1 ± 3.3 mg/g and 7.7 ± 3.3 mg/g, respectively) and 30 days (9.9 ± 4.8 mg/g and 8.4 ± 2.7 mg/g, respectively) of feeding were significantly larger (p < 0.01) than mean glycogen levels of starved specimens after 14 days (3.27 ± 1.74 mg/g and 5.37

± 3.06 mg/g, respectively) and 30 days (1.2 ± 0.5 mg/g and 1.9 ± 1.4 mg/g, respectively).

Glycogen, the primary energy reserve in bivalves, drives many important physiological processes and can be used to endure short-term exposure to anoxia, emersion, or reduced food supplies (Bayne, 1976; Gabbott, 1983; Bayne *et al.*, 1985; Hummel *et al.*, 1988). Although exposure to anoxia and emersion can be limited if unionids are relocated to suitable habitat, unionids will likely experience short-term, localized shifts in food abundance and long-term food shortages during the winter months. Normally, by accumulating glycogen when food is abundant, bivalves are able to withstand these food shortages (Gabbott, 1983; Hummel *et al.*, 1988). However, unionid survival after relocation could be greatly reduced if glycogen stores are depleted in quarantine and do not recover prior to the onset of winter. Regardless of effects on survival, decreased glycogen levels in adult bivalves also can have sublethal effects including reduced fecundity and reduced growth rates of developing offspring (Bayne, 1972; Helm *et al.*, 1973; Bayne *et al.*, 1975). Thus, the provision of adequate food resources for unionids in quarantine is pertinent to maintaining glycogen levels and enabling mussels to develop reproductively viable populations after relocation.

Results from this study indicate that relatively high cell densities of the green alga, *Neochloris oleoabundans*, in combination with *Scenedesmus* and *Ankistrodesmus*, is an adequate food resource for the maintenance of unionid glycogen stores in the short term (30 d). Currently, no information exists on the ability of unionids to digest and assimilate *Scenedesmus* and *Ankistrodesmus*, but recent studies show that unionids assimilate *Neochloris oleoabundans* with relatively high efficiency (> 50%, M. Patterson, unpub. data). Additional studies to determine which algal species are readily digested and assimilated by unionids are critical, because some algal species might not be readily digested and assimilated by certain bivalve species (Peirson, 1983). Regardless of the digestibility of a particular algal food resource, large amounts of algae will be required if management agencies hope to relocate large numbers of native mussels away from zebra mussel-infested areas. In this study, the quarantine of 300 mussels required constant culture of 750 l of living algae. Discovering ways to shorten the quarantine period would make the production of algae more feasible and decrease the time that unionids must be maintained in captivity, outside their natural habitat. Studies dealing with more efficient methods of removing zebra mussels from the shells of unionids could prove to be the best avenue for reducing the quarantine period. Ultimately, a short quarantine period along with the provision of food will improve the body condition of unionids and improve the success of future relocations.
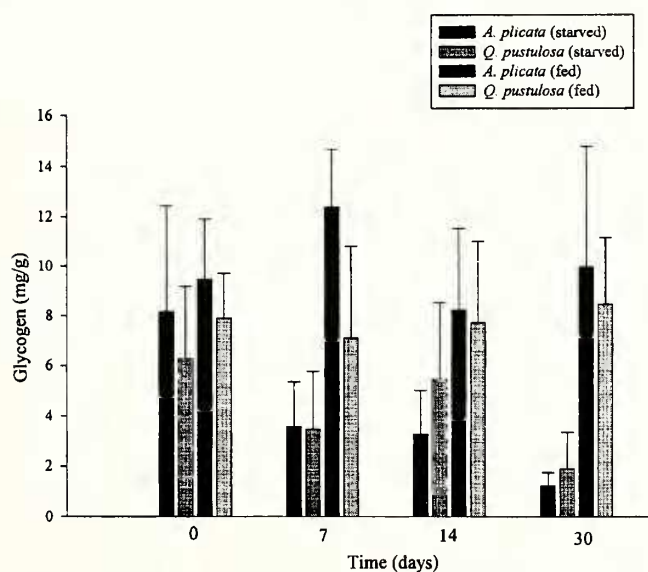

Fig. 1. Glycogen levels of *Amblema plicata* and *Quadrula pustulosa* at 0, 7, 14, and 30 d of starvation and controlled feeding in quarantine (N = 10/sampling period).

ACKNOWLEDGMENTS

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